processes

MDPI

*Article*

# Bioactive Compounds, Antioxidant Activity, and Antiproliferative Potential on Glioblastoma Cells of Selected Stone Fruit Juices

**Drazen Raucher [1,*], Mandy Rowsey [1], James Hinson [1], Ina Ćorković [2], Mary Ann Lila [3], Josip Šimunović [4] and Mirela Kopjar [2,*]**

[1] Department of Cell and Molecular Biology, University of Mississippi Medical Center, Jackson, MS 39216, USA; mrowsey@umc.edu (M.R.); jhinson@umc.edu (J.H.)
[2] Faculty of Food Technology Osijek, Josip Juraj Strossmayer University of Osijek, F. Kuhača 18, 31000 Osijek, Croatia; ina.corkovic@ptfos.hr
[3] Plants for Human Health Institute, Food Bioprocessing & Nutrition Sciences, North Carolina State University, North Carolina Research Campus, Kannapolis, NC 28081, USA; mlila@ncsu.edu
[4] Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, Raleigh, NC 27695, USA; simun@ncsu.edu
* Correspondence: draucher@umc.edu (D.R.); mirela.kopjar@ptfos.hr (M.K.)

**Abstract:** Glioblastoma presents one of the most formidable challenges in cancer treatment, remaining persistently incurable. There is a pressing need to explore less toxic alternatives, particularly natural remedies that could be applied in glioblastoma therapy. The aim of this research is to investigate the antiproliferative potential of selected stone fruit juices—tart cherry (*Prunus cerasus*), cornelian cherry (*Cornus mas*), and blackthorn (*Prunus spinosa*)—on U87-MG and GBM43 glioblastoma cells. Their effects were compared with temozolomide (TMZ), the current standard treatment. Additionally, the juices were assessed for their bioactive compounds and antioxidant potential. Unlike the other two juices, blackthorn juice did not exhibit an antiproliferative effect on U87-MG cells. However, all three juices, including blackthorn, demonstrated antiproliferative potential against TMZ-resistant GBM43 cells. Cornelian cherry exhibited an even stronger inhibitory effect than TMZ. This observation correlated with cornelian cherry being rich in iridoids, while tart cherry juice contained significant amounts of anthocyanins and proanthocyanidins. This research sheds light on the potential of cornelian cherry juice as a source of bioactive compounds with antiproliferative effects against glioblastoma cells, particularly TMZ-resistant GBM43 cells. Further research is warranted to explore the potential development of these compounds into therapeutic agents, either as single entities or in combination therapies for glioblastoma treatment.

**Keywords:** iridoids; anthocyanins; antiproliferative potential; tart cherry juice; cornelian cherry juice; blackthorn juice



**Citation:** Raucher, D.; Rowsey, M.; Hinson, J.; Ćorković, I.; Lila, M.A.; Šimunović, J.; Kopjar, M. Bioactive Compounds, Antioxidant Activity, and Antiproliferative Potential on Glioblastoma Cells of Selected Stone Fruit Juices. *Processes* **2024**, *12*, 1310. https://doi.org/10.3390/pr12071310

Academic Editor: Ibrahim M. Abu-Reidah

Received: 6 June 2024
Revised: 17 June 2024
Accepted: 21 June 2024
Published: 24 June 2024



## 1. Introduction

Over the past several decades, gliomas, the most frequent brain tumors in the adult population, have been the subject of numerous scientific studies. In recent years, increased interest has been dedicated to refining the diagnostic criteria for gliomas, particularly focusing on molecular biomarkers for categorization. This emphasis has led to the restructuring of the WHO Classification of Tumors of the Central Nervous System in 2021 [1]. According to this classification, which is based on molecular markers such as isocitrate dehydrogenase (IDH) expression and 1p/19q codeletion, adult-type diffuse gliomas are the most prevalent tumor types. These gliomas include subtypes such as astrocytoma (IDH-mutant astrocytoma), oligodendroglioma (IDH-mutant and 1p19q-codeleted), and glioblastoma (GBM) (IDH-wildtype) [1,2].

GBM stands out as one of the most challenging tumor types for treatment, and remains incurable. While angiogenesis, proliferation, and invasion are shared biological characteristics of all gliomas, GBM's invasiveness is particularly significant [3]. Despite extensive efforts in treatment, the survival rate for GBM remains low, presenting a significant obstacle. Following diagnosis, the prognosis for survival is typically limited to 13 months, with most patients succumbing within two years. While a 5-year survival rate has been achieved in clinical trials, it applies to only 4–5% of patients [4–9]. Despite the availability of various conventional treatments such as surgical resection, radiotherapy, and chemotherapy, the outcome for a majority of patients remains dismal as a result of high tumor recurrence rates [5,7,9,10].

The present standard chemotherapeutic drug for the treatment of patients with GBM is temozolomide (TMZ), known for its capability to penetrate the blood–brain barrier [11–14]. TMZ functions as an alkylating agent, inducing DNA mismatching through methylation [11]. It also has a significant part in halting cell progression at the G2/M phase, and triggering apoptosis in cancer cells [11,13]. Despite these mechanisms, the survival rate of patients remains very low due to the development of increased resistance to the drug [11]. Consequently, there is a strong interest in identifying and developing alternative strategies to achieve greater efficacy in reducing tumors and improving patient survival rates.

More recently, research focus has been directed to novel therapeutic interventions like the utilization of micronutrients [3], and the pursuit for active ingredients from natural sources which can be used for the prevention and treatment of malignant tumors is gaining considerable attention [15,16]. Over the years, plant-derived compounds have demonstrated a remarkable ability to inhibit the growth and development of cancer cells. These substances can be used to achieve inhibition of the proliferation of cells by various mechanisms such as the initiation of apoptosis, autophagy, and the blocking of cancer cells at different phases of the cell cycle [6]. In addition, many of these compounds have demonstrated the ability to deactivate the signaling pathways which are generally activated or conversely activate the signaling pathways which are generally deactivated in cancer cells [17–21]. One very important consideration is that most plant-derived compounds are considered safe for human consumption due to their minimal harmful effects. Consequently, it is believed that plant-derived anticancer drugs may cause mild or even no side effects on the overall health of the cancer patients [17,21,22]. In order to achieve the improvements of overall patient prognosis, besides the inhibition of the growth of cancer cells and the prevention of the development of recurrent tumors, these molecules need to possess the ability to pass through the blood–brain barrier [17]. In most cases, cancer suppression results from apoptosis or the programmed death of cells [23,24]. The proliferation of preneoplastic or neoplastic cells tends to be more significant than that of normal cells, making the induction of apoptosis or arrest of the cell cycle crucial for inhibiting the stimulation and progression of carcinogenesis, and ultimately for eliminating genetically damaged, preinitiated, or neoplastic cells from the organism [23,24]. Preinitiated cells refer to those that have undergone initial genetic changes or alterations that predispose them to malignancy but have not yet progressed to the stage of being fully initiated cancer cells. These cells represent an early stage of transformation and are potentially capable of developing into cancerous cells if not eliminated or halted through mechanisms such as apoptosis or cell cycle arrest. Generally, polyphenols are known for their anticancer effect, with anthocyanins known for apoptotic effects in human cancer cells [23–25], so their natural sources could potentially be used in cancer treatments. In a recent review of Ndongwe et al. [26], it was elaborated that iridoids, a large group of natural compounds, have the ability to generate conjugates with other drugs such as anticancer, antidiabetic, antileishmanial, and antimalarial drugs. These conjugations can result in synergistic effects and have the potential to increase drug efficiency. Additionally, the authors noted the importance of the full exploration of the role of iridoids in identifying less expensive and less toxic alternative/adjuvant cancer drugs [26].

The goal of this research was to assess the antiproliferative effects of specific stone fruit juices (tart cherry, cornelian cherry, and blackthorn) on two glioblastoma cell lines, U87-MG and GBM43. These two cell lines were selected since they are frequently used in cancer research on the glioblastoma multiforme. U87-MG cells are capable of generating tumors in experimental animals so they are frequently utilized as a model system for the investigation of glioblastoma. They are applied to the investigation of diverse aspects of the biology of glioblastoma, including growth of tumor, its invasiveness, and its response to selected therapies. Consequently, these cells are utilized in the testing of various drugs and preclinical studies [27–29]. The source and manipulation of the cells are key factors in determining the specific properties of GBM43 cells. They can easily share attributes that are mutual to glioblastoma cells, like accelerated proliferation and invasiveness. Mentioned cells are also utilized to acquire insights into glioblastoma biology, as well as for testing potential therapeutic interventions [30–32]. Additionally, a very important feature of these cells is that U87-MG glioblastoma cells are TMZ-sensitive, while GBM43 cells are TMZ-resistant [14]. Selected stone fruit juices' antiproliferative effects on glioblastoma cells were compared with those of TMZ, the present standard drug for glioblastoma treatment. Furthermore, the juices were analyzed for the contents of their bioactive compounds, total polyphenols and proanthocyanidins, as well as antioxidant potential.

## 2. Materials and Methods

### 2.1. Chemicals

Trolox, 4-dimethylaminocinnamaldehyde, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), gallic acid, chlorogenic acid, ellagic acid, rutin, (-)-epicatechin, and loganic acid were acquired from Sigma-Aldrich (St. Louis, MO, USA). Analytical standards of anthocyanins (cyanidin-3-glucoside, cyanidin-3-rutinoside), neochlorogenic acid, and hyperoside were acquired from Extrasynthese (Genay, France). From T.T.T. (Sveta Nedelja, Croatia), sodium carbonate was obtained, while potassium persulfate and Folin-Ciocalteu reagent were obtained from Kemika (Zagreb, Croatia). Orthophosphoric acid (HPLC-grade) was acquired from Fisher Scientific (Loughborough, UK), and methanol (HPLC-grade) from J.T. Baker (Deventer, The Netherlands). Neocuproine, cupric chloride, and 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) were acquired from Acros Organic (Geel, Belgium). Thiazolyl blue tetrazolium bromide (98%) (2-(3,5-diphenyltetrazol-2-ium-2-yl)-4,5-dimethyl-1,3-thiazole;bromide), Temozolomide (3-methyl-4-oxoimidazol[5,1-d][1,2,3,5]tetrazine-8-carboxamide), DMEM media (with L-glutamine, 4.5 g/L glucose and sodium pyruvate), solution of penicillin/streptomycin (10,000 U/mL penicillin, 10,000 µg/mL streptomycin in 0.85% NaCl), and 0.25% trypsin/0.1% EDTA were obtained from ThermoFisher Scientific (Waltham, MA, USA). Glioblastoma U87-MB and GBM43 cells were obtained from Mayo Clinic (Rochester, MN, USA) while fetal bovine serum was acquired from R&D Systems (Flowery Branch, GA, USA).

### 2.2. Preparation of Stone Fruit Juices

Stone fruits, namely tart cherry (*Prunus cerasus*), cornelian cherry (*Cornus mas*), and blackthorn (*Prunus spinosa*) fruits were grown near Varaždin (Croatia) at location 46°18′39.7″ N 16°32′67.7″ E. After the collection of these fruits (approximately 2 kg), they were first washed and pitted, and afterward pressed. The resulting juices were filtered through cheesecloth, followed by two min thermal treatment (300 mL of each juice) at 90 °C for inactivation of the naturally present enzymes and vegetative bacteria which could cause the degradation and spoilage of juices.

### 2.3. Evaluation of Total Polyphenols, Proanthocyanidins, and Monomeric Anthocyanins of Stone Fruit Juices

To determine the total polyphenols in the juices, the method of Singleton and Rossi [33] was applied. A total of 10 mL of Folin-Ciocalteu reagent (7.5%) was mixed with 0.2 mL of

diluted juice and 1.8 mL demineralized water. A total of 8 mL of sodium carbonate solution (7.5%) was added to this mixture and it was kept for 120 min in a dark place before the reading of the absorbance at 765 nm took place. Results were presented as grams of gallic acid equivalents per L of juice (g GAE/L), so the calibration curve was generated with gallic acid. Readings were performed on a UV/Vis spectrophotometer (Cary 60 UV-Vis, Agilent Technologies, Santa Clara, CA, USA).

The concentration of proanthocyanidins was evaluated by the 4-(dimethylamino) cinnamaldehyde (DMAC) method [34]. Then, 1 mL of DMAC reagent was mixed with diluted juice and acidified ethanol. The mixture was kept for 30 min in a dark place before the reading of absorbance at 640 nm took place. The concentration of proanthocyanidins was presented as mg of procyanidin B2 equivalent per L of the juice (mg B2E/L), so the calibration curve was generated for procyanidin B2.

The pH differential method was applied to estimate the monomeric anthocyanins [35]. Two buffers (pH 1–0.025 M KCl and pH 4.5–0.4 M sodium acetate) were prepared to perform the analysis. A total of 2.8 mL of each buffer was mixed with 0.2 mL of the diluted juice. Mixtures were kept for 15 min in a dark place before the reading of absorbance at 515 nm and 700 nm took place. Concentrations of monomeric anthocyanins were presented as mg of cyanidin-3-glucoside per L of the juice (mg cyanidin-3-glucoside/L).

### *2.4. Evaluation of Antioxidant Activity of Stone Fruit Juices*

To evaluate the antioxidant potential of the juices, ABTS, DPPH, FRAP, and CUPRAC assays were applied. The results of antioxidant activities were presented as μmol of Trolox equivalents per 100 mL of juice (μmol TE/100 mL), so calibration curves for all assays were generated using Trolox.

#### 2.4.1. ABTS Assay

The ABTS assay was performed according to Arnao et al. [36]. A total of 3.2 mL of ABTS reagent was mixed with 0.2 mL of the diluted juice. The mixture was kept for 95 min in a dark place before the reading of absorbance at 734 nm took place.

#### 2.4.2. DPPH Assay

For the DPPH method [37], 3 mL of DPPH solution was mixed with 0.2 mL of the diluted juice. The mixture was kept for 15 min in a dark place before the reading of absorbance at 517 nm took place.

#### 2.4.3. FRAP Assay

The ferric-reducing ability was evaluated according to Benzie and Strain [38]. A total of 3 mL of FRAP reagent was mixed with 0.2 mL of the diluted juice. The mixture was kept for 30 min in a dark place before the reading of absorbance at 593 nm took place.

#### 2.4.4. CUPRAC Assay

For the evaluation of cupric ion-reducing antioxidant capacity, the CUPRAC assay was utilized [39]. Copper chloride, neocuproine, and ammonium acetate buffer (pH 7) solution were mixed to a ratio of 1:1:1. To this mixture 0.2 mL of the diluted juice was added. The mixture was kept for 30 min in a dark place before the reading of absorbance at 450 nm took place.

### *2.5. Preparation of Stone Fruit Juices for High Performance Liquid Chromatography (HPLC)*

Prior to HPLC analysis, to exclude impurities samples went through solid-phase extraction using commercial sorbent, Strata$^{TM}$-X 33 μm Polymeric Reversed Phase from Phenomenex (Torrance, CA, USA). Preconditioning with methanol (HPLC-grade) of cartridges was conducted after their insertion in a vacuum manifold which was operating at room temperature. Then, acetic acid solution (1% in water) was added, followed by the addition of the sample. The sample was dripping, and formed a compact ring in

the cartridges. After the cartridges were dried, elution of bioactives was conducted with methanol [40,41]. The eluents were gathered and used for injection into the HPLC system.

### 2.6. Evaluation of Bioactive Compounds of Stone Fruit Juices Using Reversed Phase HPLC

The detection and quantification of individual bioactive compounds in juices was performed by HPLC (Agilent HPLC system 1260 Infinity II, Santa Clara, CA, USA). The whole system was composed of a quaternary pump, a vial sampler, DAD detector (which was recording spectra in the interval from 190 to 600 nm), and column (Poroshell 120 EC C-18, 4.6 × 100 mm, 2.7 µm). Buljeta et al. [42] have previously described the method. Orthophosphoric acid (0.1%) was used as a mobile phase A, and methanol was used as a mobile phase B. Both mobile phases were HPLC-grade. The injected volume of sample was set at 5 µL, and the flow rate was set at 1 mL/min. For the quantification of bioactive compounds, calibration curves for standards of anthocyanins (cyanidin-3-glucoside, cyanidin-3-rutinoside), hyperoside, rutin, gallic acid, ellagic acid, chlorogenic acid, neochlorogenic acid, (-)-epicatechin, and loganic acid were generated with a linearity of $R^2 > 0.99$. Anthocyanins were recorded at 520 nm, rutin and hyperoside at 360 nm, ellagic acid at 250 nm, gallic acid and (-)-epicatechin at 280 nm, neochlorogenic acid and chlorogenic acid at 320, and loganic acid at 245 nm. Cyanidin-3-glucosylrutinoside and derivate of pelargonidine-3-glucoside were expressed through cyanidin-3-glucoside, and cornuside through loganic acid. Concentrations of bioactives were presented as mg of bioactive compound per L of the juice (mg/L).

### 2.7. Evaluation of Antiproliferative Effects of Stone Fruit Juices on U87-MG and GBM43 Glioblastoma Cells

Two glioblastoma cell lines, U87-MG and GBM43, were used to assess the antiproliferative effects of selected stone fruit juices. Glioblastoma U87-MG cells were derived from a 44-year-old female patient, and GBM43 cells from a male patient. These cell lines were obtained from the American Type Culture Collection (ATCC) (U87-MG: ATCC® HTB-14™, GBM43: ATCC® CRL-3308™), ensuring their authenticity and reliability. The selected cells were cultured in 96 well plates. The cells were treated with stone fruit juices after 24 h. Fruit juices were applied in amounts of 1%, 2%, or 3% of the culture medium (DMEM—10% fetal bovine serum—1% penicillin/streptomycin). Positive and negative controls were also prepared. As the positive control for inducing cell death, cells treated with TMZ were used, while as the negative control untreated cells were used. The treatment period lasted for 3 days, after which the survival of glioblastoma cells was evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) method [43]. The method is based on the measurement of the metabolic activity of living cells. During reaction, MTT reagent is converted from yellow to purple due to the formation of formazan crystals by viable cells. Absorbance of the obtained purple formazan crystals was read and used for their quantification. An indication of greater cell viability is higher absorbance, reflecting the effectiveness of the treatment. The percentage of survival of glioblastoma cells for each treatment group was calculated according to following equation:

$$\text{Percentage of survival} = (A_{TS}/A_{UTS}) \times 100$$

where $A_{TS}$ was absorbance of cells that were treated, and $A_{UTS}$ the absorbance of untreated cells.

### 2.8. Statistical Analysis of the Obtained Results

For each assay, all samples were estimated in triplicate. The obtained results for bioactives and antioxidant activities of juices were compared using analysis of variance (ANOVA) and Fisher's least significant difference (LSD), with the significance defined as $p < 0.05$. Statistical analyses were conducted by the software program STATISTICA 13.1 (StatSoft Inc., Tulsa, OK, USA). The obtained results for antiproliferative effects of stone fruit juices on chosen glioblastoma cells were plotted and statistically analyzed using

one-way analysis of variance (ANOVA) to evaluate the significant differences in survival percentages between groups, with the significance defined as $p < 0.05$, $p < 0.01$, $p < 0.001$. For this purpose, GraphPad Prism version 10.1.2. was applied.

## 3. Results

### 3.1. Bioactive Compounds and Antioxidant Potential of Stone Fruit Juices

Results of the determinations of total polyphenols, monomeric anthocyanins, proanthocyanidins, and antioxidant activities of selected stone fruit juices are presented in Table 1. Blackthorn and tart cherry juices had similar total polyphenol contents (approximately 2.6 g/L), which were higher than those of cornelian cherry juice (1.81 g/L). Tart cherry juice contained three times the amount of monomeric anthocyanins than blackthorn juice did (468.36 mg/L vs. 152.25 mg/L), while cornelian cherry juice had the lowest content of monomeric anthocyanins (15.51 mg/L). The highest content of proanthocyanidins was in tart cherry juice (1254.99 mg/L), whereas for the other evaluated juices this was 306.9 mg/L and 21.56 mg/L in blackthorn and cornelian cherry juices, respectively. Four methods were used to measure antioxidant activity, namely the DPPH, ABTS, FRAP, and CUPRAC methods. The highest antioxidant activity measured by the DPPH method was in cornelian cherry (9.08 µmol/100 mL), followed by blackthorn (8.40 µmol/100 mL) and tart cherry (6.51 µmol/100 mL) juice. However, the application of the ABTS method indicated that all juices had similar antioxidant activities (approximately 18 µmol/100 mL). Results of antioxidant activity obtained by the FRAP method indicated that blackthorn juice had the highest antioxidant potential, while cornelian cherry had the lowest (1.99 µmol/100 mL and 1.51 µmol/100 mL, respectively). The same trend was observed for the CUPRAC method (116.56 µmol/100 mL and 71.59 µmol/100 mL for blackthorn and cornelian cherry juices, respectively).

**Table 1.** Total polyphenols (TP), monomeric anthocyanins (ANT), proanthocyanidins (PAC), and antioxidant activity (µmol/100 mL) of stone fruit juices.

| Parameters | Juice | | |
|---|---|---|---|
| | Tart Cherry | Cornelian Cherry | Blackthorn |
| TP (g/L) | 2.58 ± 0.05 [a] | 1.81 ± 0.01 [b] | 2.69 ± 0.02 [a] |
| ANT (mg/L) | 468.36 ± 3.87 [a] | 15.51 ± 0.51 [c] | 152.25 ± 0.13 [b] |
| PAC (mg/L) | 1254.99 ± 24.61 [a] | 21.56 ± 1.13 [c] | 306.90 ± 4.75 [b] |
| DPPH | 6.51 ± 0.06 [c] | 9.08 ± 0.15 [a] | 8.40 ± 0.05 [b] |
| ABTS | 17.65 ± 0.26 [a] | 17.43 ± 0.32 [a] | 18.13 ± 0.37 [a] |
| FRAP | 1.72 ± 0.03 [b] | 1.51 ± 0.00 [c] | 1.99 ± 0.02 [a] |
| CUPRAC | 91.56 ± 0.77 [b] | 71.59 ± 0.08 [c] | 116.56 ± 0.19 [a] |

Values marked with various letters (a–c) in the same row are significantly different at $p < 0.05$.

HPLC analysis was utilized for the evaluation of bioactive compounds in chosen juices, and the results are presented in Table 2. In all juices, individual polyphenols were found, while iridoids were found only in cornelian cherry juice. The most abundant polyphenols in tart cherry juice were anthocyanins, cyanidin-3-rutinoside, and cyanidin-3-glucosylrutinoside (420.71 mg/L and 342.29 mg/L, respectively). Chlorogenic and neochlorogenic acids were also found in high concentrations (156.42 mg/L and 102.92 mg/L, respectively). Additionally, epicatechin (40.17 mg/L) and rutin (46.54 mg/L) were found. Blackthorn juice had the highest concentration of neochlorogenic acid (693.69 mg/L). It also contained anthocyanins (cyanidin-3-rutinoside and cyanidin-3-glucoside in concentration of 73.28 mg/L and 23.36 mg/L, respectively) and hyperoside (19.74 mg/L). Cornelian cherry juice contained cyanidin-3-glucoside (7.03 mg/L) and derivate of pelargonidin (7.96 mg/L). Also, gallic (23.93 mg/L), chlorogenic (10.87 mg/L), and ellagic (2.05 mg/L)

acids were found. In addition to polyphenols, iridoids were identified in cornelian cherry juice, and they were the prevalent bioactives in this juice. Two iridoides were found: loganic acid, which was the dominant one (1285.82 mg/L), and cornuside (76.88 mg/L).

**Table 2.** Bioactive compounds (mg/L) found in stone fruit juices by HPLC analysis.

| Bioactive Compounds | Juice | | |
|---|---|---|---|
| | Tart Cherry | Cornelian Cherry | Blackthorn |
| *Individual polyphenols* | | | |
| Cyanidin-3-glucoside | ND | 7.03 ± 0.08 [b] | 23.36 ± 0.27 [a] |
| Cyanidin-3-rutinoside | 420.71 ± 13.74 [a] | ND | 73.28 ± 0.09 [b] |
| Cyanidin-3-glucosylrutinoside | 342.29 ± 8.47 | ND | ND |
| Pelargonidine * | ND | 7.96 ± 0.03 | ND |
| Hyperoside | ND | ND | 19.74 ± 0.16 |
| Rutin | 46.54 ± 0.94 | ND | ND |
| (-)-epicatechin | 40.17 ± 1.60 | ND | ND |
| Gallic acid | ND | 23.93 ± 0.16 | ND |
| Ellagic acid | ND | 2.05 ± 0.09 | ND |
| Chlorogenic acid | 156.42 ± 3.35 [a] | 10.87 ± 0.34 [b] | ND |
| Neochlorogenic acid | 102.92 ± 2.04 [b] | ND | 693.69 ± 12.66 [a] |
| *Iridoides* | | | |
| Loganic acid | ND | 1285.82 ± 17.85 | ND |
| Cornuside | ND | 76.88 ± 3.52 | ND |

*—derivate; ND—not detected. Values marked with various letters (a, b) in the same row are significantly different at $p < 0.05$.

### 3.2. Antiproliferative Effects on U87-MG and GBM43 Cells of Stone Fruit Juices

The inhibition of the proliferation of glioblastoma U87-MG and GBM43 cell lines in response to stone fruit juices is presented in Figures 1 and 2. Their potential for inhibiting the proliferation of these cell lines was compared with the potential of TMZ, which is the standard chemotherapeutic drug for treating select tumor cell lines. The juices were applied at concentrations of 1%, 2%, and 3%. When TMZ was applied, the percentage of survival of glioblastoma U87-MG cells was 61.4%. Blackthorn juice did not exhibit an antiproliferative effect on these glioblastoma cells, while tart cherry and cornelian cherry juices showed slight effects. The survival rate ranged from 81% to 92% when tart cherry juice was applied, and from 71% to 82% when cornelian cherry juice was applied.

When TMZ was used for the inhibition of growth of GBM43 glioblastoma cells, their survival rate was 73.7%. All three juices demonstrated the potential for inhibition of GBM43 cells, however only cornelian cherry juice showed a good potency. Percentages of survival were 78.2%, 56.9%, and 41.8% when 1%, 2% or 3% of cornelian cherry juice was used for the inhibition of cells. The other two juices had lower potency for the inhibition of GMB43 cells than TMZ and cornelian cherry juice did. Survival rates ranged from 69% to 88% when tart cherry juice was applied, and from 76% to 87% when blackthorn juice was applied.

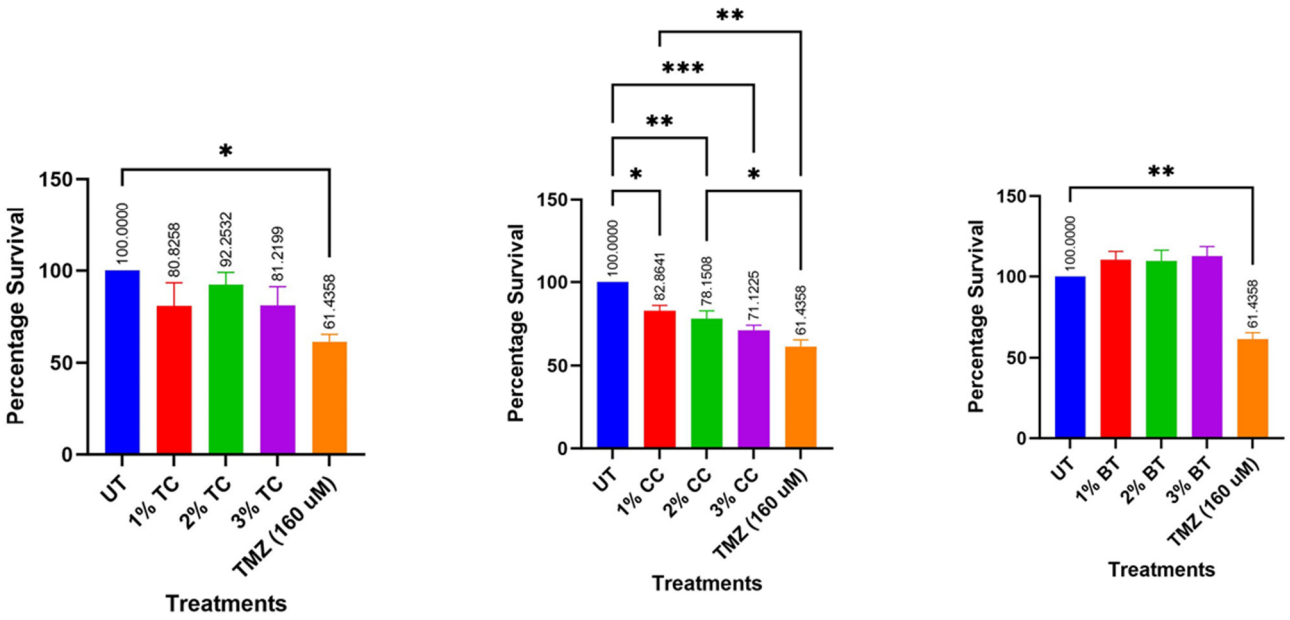


**Figure 1.** Antiproliferative effect of investigated stone fruit juices on U87-MG glioblastoma cells. (*p* values * < 0.05, ** < 0.01, *** < 0.001); UT—control; TMZ—Temozolomide; TC—tart cherry juice; CC—cornelian cherry juice; BT—blackthorn juice.

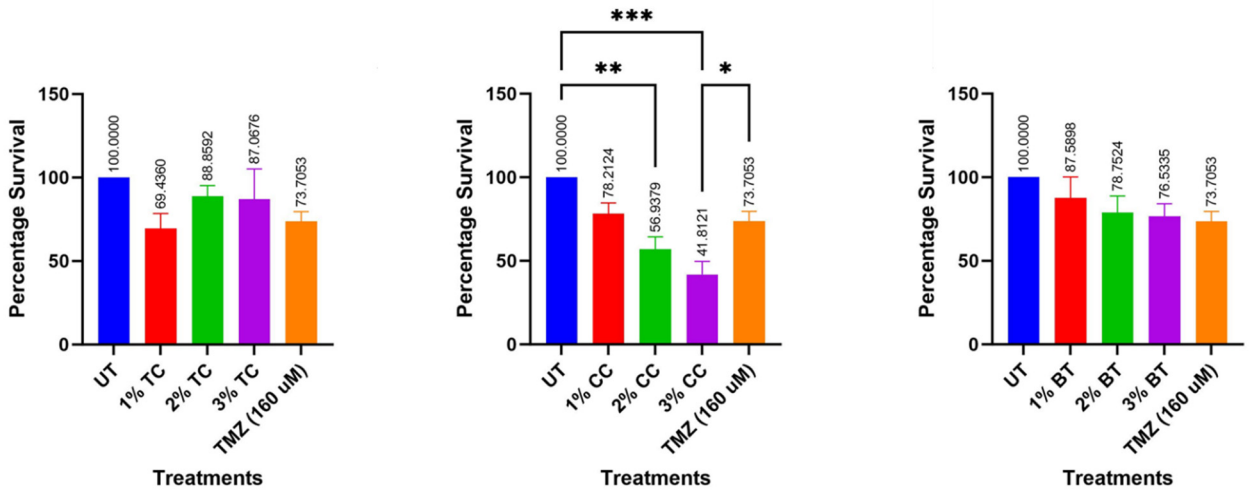


**Figure 2.** Antiproliferative effect of investigated stone fruit juices on GBM43 glioblastoma cells. (*p* values * < 0.05, ** < 0.01, *** < 0.001); UT—control; TMZ—Temozolomide; TC—tart cherry juice; CC —cornelian cherry juice; BT—blackthorn juice.

## 4. Discussion

The antiproliferative effects of selected stone fruit juices on U87-MG and GBM43 glioblastoma cells were compared with TMZ, the current standard chemotherapeutic drug of choice for the treatment of patients with glioblastoma. These two types of cell are commonly employed in glioblastoma research due to their ability to provide insights into the biology of glioblastoma, cancer cell growth, invasiveness, and suitability for testing potential therapeutic interventions [27–32]. As previously mentioned, there has been a growing interest in exploring active ingredients from natural sources for the prevention and treatment of malignant tumors [15,16]. Differences observed among the chosen stone fruit juices were in their profiles of bioactive compounds, their concentrations, and antioxidant activities. While the tart cherry and blackthorn juices had similar contents of total polyphenols, tart cherry juice had a considerably higher content of monomeric anthocyanins and

proanthocyanidins. Tart cherry and cornelian cherry juices exhibited a slight potential for the inhibition of growth of glioblastoma U87-MG cells, however, in both cases it was lower than the one achieved with TMZ. Blackthorn juice was the only one among the tested juices to exhibit no potential for inhibition. All three juices showed a potential for inhibition of growth of glioblastoma GBM43 cells. Tart cherry and blackthorn juices had a similar or lower effect on the growth of GBM43 cells in comparison to TMZ. Only cornelian cherry juice, when applied in amounts of 2% and 3%, had a higher antiproliferative effect than TMZ. This positive influence of juices on the inhibition of glioblastoma cells can be ascribed to bioactive compounds which have been identified. In a previous study, we examined the impact of berry juices, namely raspberry, dwarf elderberry, and wild blackberry on the inhibition of growth of U87-MG and GMB46 glioblastoma cells [44], and it was demonstrated that the juices had the potential to inhibit both glioblastoma cells. It was determined that only wild blackberry juice exhibited higher potential to inhibit the growth of U87-MG cells compared to TMZ, while all berry juices demonstrated higher potency in inhibiting TMZ-resistant GBM43 cells [44]. This positive effect of berry juice is attributed to the polyphenols present in these juices, particularly anthocyanins. Although previous research indicated that berry juices with the highest concentration of anthocyanins had the highest potency in inhibiting the growth of both glioblastoma cells, the present study did not observe the same trend. Tart cherry juice had the highest anthocyanins concentration, but cornelian cherry had the highest potency in inhibition of growth of both cells, especially TMZ-resistant GMB43 cells. Cornelian cherry juice generally had the lowest content of polyphenols, thus its positive effect on glioblastoma cells could be ascribed to its possession of iridoids, especially loganic acid, bioactives that were not identified in the other two juices. Some studies showed the potential of iridoids as new anti-tumor drugs since they can prevent replication of DNA in cancer cells [21,26,45]. They specifically target neurotoxicity and oxidative stress, which is necessary in the treatment of disease through the improvement of antioxidant defenses and blocking cascades [26,46–49]. Significant biological activity of iridoids is assigned to the suppression of the expression of multiple important pro-inflammatory proteins, consequently achieving a variety of anti-inflammatory actions [26,50]. It was determined that anthocyanins and iridoids present in cornelian cherry fruits can modulate the redox system and pro-inflammatory cytokines [51]. Loganic acid inhibited the proliferation, invasiveness, and cellular migration of hepatocellular tumor cells by regulating the levels of protein of mesenchymal markers in CXCL12-treated cells. Additionally, it eliminated the MMP-9/2 gelatinolytic activity. It was suggested that loganic acid could be an anti-metastatic agent since it can suppress metastasis and epithelial mesenchymal transition processes throughout the reduction of expression of MnSOD in hepatocellular carcinoma cells [52]. Nano carriers of bioactive compounds of cornelian cherries also had the potential to inhibit the colorectal cancer cell line HT-29 by arresting the proliferation of cells in the G1 phase and causing its apoptosis [53]. Sweroside, an iridoid, also showed inhibitory effects on the growth of the U251 glioblastoma cells by causing the apoptotic death of cells. This was accompanied by upregulation of apoptotic proteins such as caspase 3 and 9, and Bax expressions. In addition, it induced arrest at the G0/G1 phase of the cell cycle and the JNK/p38 MAPK signal pathway [21].

The influence of tart cherry juice on glioblastoma cells could be attributed to high anthocyanins concentration, but also to high proanthocyanidin content. Anthocyanins have also been emphasized by other researchers as polyphenols important to the inhibition of the growth of different cancer cells. It was determined that they can be more efficient than other flavonoids for the inactivation of direct cell growth [23]. Petunidin, cyanidin, and delphinidin anthocyanidins could potentially cause the inhibition of glioblastoma cancer cells by affecting plasminogen activation and, through that, the inhibition of the migration of cancer cells. Their structure, more precisely the number of OH-groups on the B-ring, were highlighted as a cause for this action. Petunidin and cyanidin forms possess two OH-groups, and their inhibitory effect was 48%, while delphinidin had an inhibitory effect of 83% due to the possession of three OH-groups [9]. Regardless of their structure, when cyanidin and

delphinidin were tested for their antiproliferative and apoptotic impact on MCF7 cell lines (human breast cancer), it was determined that cyanidin was more efficient [54]. Regarding structure, glycosylation can be a very important factor. Glycosides of aglycons could be more effective due to their ability to hinder glucose transport that results in the inhibition of energy metabolism, which, in turn, can cause mitochondrial damage and apoptosis of tumor cells [55]. Cyanidin-3-glucoside and cyanidin-3-glucosylrutinoside were found in high concentration in tart cherry juice, while in blackthorn juice cyanidin-3-glucoside and cyanidin-3-rutinoside were found, so those compounds were probably responsible for the positive influence of these juices on the proliferation of glioblastoma cells. Cyanidin-3-rutinoside and cyanidin-3-glucoside (fraction of mulberry anthocyanins) have been tested against human lung cancer cells, and it was found that they have an inhibitory influence on the migration as well as on the invasion of the highly metastatic A549 cells. The mechanism by which those anthocyanins inhibited lung cancer cells included the decrease in the expression of matrix matalloprotinase-2 (MMP-2) and urokinase-plasminogen activator (u-PA), and the increase in the expression of the tissue inhibitor of matrix matalloprotinase-2 (TIMP-2) and plasminogen activator inhibitor (PAI) [56]. Kang et al. [57] showed that anthocyanins and their aglycone, cyanidin, present in tart cherries, significantly diminished tumor development in the cecum of $Apc^{Min}$ mice. In addition, these compounds caused the direct inhibition of the growth of HT 29 and HCT 116 cell lines (human colon cancer), with the aglycone cyanidin having much higher potential in comparison to the anthocyanin glycosides. Anthocyanin fractions of extracts of Mexican wild blackberries mostly possessed cyanidin-3-rutinoside and cyanidin-3-glucoside, and these extracts caused the initiation of apoptosis in C6 and RG2 cell lines. The inhibition of the C6 cell lines with these extracts was through the G0/G1 phase [4].

Procyanidins isolated from grape seeds have been one of the natural materials used to evaluate the effect of bioactives on glioblastoma cells. It was observed that they significantly inhibited the growth of glioblastoma by inducing G2/M arrest and decreasing mitochondrial membrane potential in U-87 cells. In addition, in these cells procyanidins caused the death of the non-apoptotic cell phenotype resembling paraptosis. They also caused the inhibition of U-87 cells by influencing a G-protein-coupled receptor, specifically the formyl peptide receptor, which is involved in the invasion and metastasis of tumor cells [58]. Cranberry proanthocyanidins and flavonoid-rich extract were tested against colon carcinoma (HT-29), glioblastoma multiforme (U87), and androgen-independent prostate carcinoma (DU145). It was found that the inhibition of these different cancer cells was achieved. The proanthocyanidin fraction was more effective than the flavonoid fraction at inducing the inhibition of glioblastoma cells in the G1 cell cycle in a time- and dose-dependent manner [59].

Karakas et al. [60] investigated the cytotoxicity of methanol extract of blackthorn fruit on glioblastoma cancer (LN229, T98G, U87) cell lines, and pancreatic cancer (PANC-1, ASPC-1) cell lines. In GBM cell lines, a decrease in cell viability was observed. However, by calculating $IC_{50}$ values it was determined that the highest concentration was necessary for the inhibition of U87 cells. $IC_{50}$ values were 5.245 mg/mL, 5.459 mg/mL, and 9.777 mg/mL of methanol extracts of blackthorn fruit for LN229, T98G, and U87 GBM cells, respectively. On the other hand, no effect was detected on either of the investigated pancreatic cancer cells [60].

It is important to consider that the presence of other phenols, as well as their concentrations and ratios with other phenolics, can significantly influence the availability of free-binding sites that could interact with glioblastoma cells. Interactions between compounds can result in achieving synergistic or antagonistic effects, as was also evident from the results of the determination of antioxidant activities.

Both the ABTS and DPPH methods are based on the reaction of H-atom donors with corresponding radicals, $ABTS^{\cdot+}$ and $DPPH^{\cdot}$. From our results, it is evident that higher antioxidant activity was achieved by the ABTS method, and there was no difference between samples in contrast to DPPH method. $ABTS^{\cdot+}$ is characterized by the reaction with

any hydroxylated aromatics, irrespective of their actual antioxidative potential, which also includes OH-groups that do not contribute to the antioxidation [61]. $DPPH^{\cdot}$ is characterized by higher selectivity in comparison to $ABTS^{\cdot+}$ in the reactions with H-donors. As opposed to $ABTS^{\cdot+}$, $DPPH^{\cdot}$ does not react with flavonoids, which in B-ring have no OH-groups, or with aromatic acids, which have only one OH-group [61]. Even though the cornelian cherry juice contained the lowest concentration of polyphenols, it had the highest antioxidant activity estimated by the DPPH assay, probably due to the high concentration of loganic acid. It was observed that loganic acid had remarkable antioxidant activity in terms of DPPH scavenging [62], so its high concentration in cornelian cherry juice compensated antioxidant activity. The other two methods, FRAP and CUPRAC, are based on the reduction of metal ions by the action of antioxidants. The mechanism of the first one involves the reduction of the ferric ion ($Fe^{3+}$)-ligand complex to ferrous ($Fe^{2+}$) complex [63], and the second one the reduction of cupric ($Cu^{2+}$) to cuprous ion ($Cu^{+}$) [64]. It is evident from the results of antioxidant activity determined by these methods that blackthorn juice had the highest antioxidant potential, even though the tart cherry juice contained considerably more anthocyanins and proanthocyanidins. This can be explained by the antagonistic phenomena that are the consequence of interactions between the phenolic compounds. It is well known that the number as well as position of OH-groups and $OCH_3$-groups on the phenolic rings predominantly affect the antioxidant potential of individual phenolic compounds. Nevertheless, the antioxidant potential of combined compounds, i.e., phenolic mixtures, is a complex outcome of different parameters, like intramolecular interactions, concentration of compounds, dissociation, ionization, matrix interference, etc. Consequently, the antioxidant potential of the complete mixture can be additive, synergistic, or antagonistic [65–67].

Generally, flavonoids (including anthocyanins) are mostly present in the form of metabolites in circulation since they are subjected to degradation reactions throughout the digestive system [68]. Consequently, anthocyanin forms, both intact and altered, can be detected in plasma. The most important altered forms are the corresponding phenolic acids and aldehydes, and various conjugates (for example methyl, sulfate, and glucuronyl conjugates) [69]. Nevertheless, studies have shown that flavonoids (including anthocyanins), as well as their altered forms (metabolites), may be detected in brain tissue due to their ability to pass across the blood–brain barrier [70], one of the obstacles in the efficient treatment of glioblastoma. Considering iridoids detected in cornelian cherry juice, it was determined that loganic acid was digested (in the experimental conditions) in contrast to cornuside. The fact that cornuside was present of in the colon fraction from gastrointestinal digestion in vitro makes this iridoid potentially bio-accessible [71]. Regarding their permeability through blood–brain barrier, it was determined that some iridoids have this ability. Valtrate, an iridoid component, has the ability to pass through the blood–brain barrier [72]. It was observed that valtrate had a potential antitumor activity against GBM cells in vitro and in vivo. Next to the inhibition of the proliferation, invasion, and migration of GBM cells, it also caused the induction of apoptosis in these cells [15]. Some other iridoids, such as gardenoside and agnuside, can also pass across the blood–brain barrier, which may be used for therapeutic purposes [73,74]. A variety of other iridoids can potentially also be used as therapeutic agents for intracerebral targeting because of their ability to pass across the blood–brain barrier. Even so, the key issue is the low content distribution within the brain, since these compounds go through rapid absorption as well as elimination in vivo, and are widely distributed to tissues and organs. Consequently, they have few biological benefits when they are orally administered [75].

Generally, delivering drugs to glioblastoma cells presents an extremely challenging objective for research and development. Considerable endeavor has been dedicated to developing effective delivery systems with the purpose of overcoming the heterogeneity of tumor cells (both molecular and cellular), their infiltrative nature, and the blood–brain barrier. Our results can contribute to the formulation of delivery systems, as some flavonoids and iridoids have demonstrated the ability to cross the blood–brain barrier. With this property and their potential to inhibit the growth of glioblastoma cells, these bioactives

have the potential to be individually incorporated into delivery systems or combined with other natural bioactives and/or used in combination with TMZ to enhance its effects. This possibility should also be explored in future investigations.

## 5. Conclusions

The results of this study emphasized the diversity in composition of bioactive compounds and antioxidant capacities of chosen stone fruit juices (tart cherry, cornelian cherry, and blackthorn) and their potential for the inhibition of glioblastoma U87-MG and GBM43 tumor cells. While blackthorn juice did not inhibit growth of U87-MG cells, the other two juices did exhibit some potential to do so. TMZ-resistant GBM43 cells were affected by all three juices, but only the cornelian cherry juice showed higher potency than TMZ. For future studies, higher concentration levels of juice inclusion in the cell growth media should also be considered, as well as combinations of natural bioactives and their combinations with TMZ.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr12071310/s1, Figure S1: HPLC chromatogram at 520 nm of tart cherry juice (1—cyanidin-3-glucosylrutinoside; 2—cyanidin-3-rutinoside). Figure S2: HPLC chromatogram at 360 nm of tart cherry juice (3—rutin). Figure S3: HPLC chromatogram at 320 nm of tart cherry juice (4—neochlorogenic acid; 5—chlorogenic acid). Figure S4: HPLC chromatogram at 210 nm of tart cherry juice (6—(-)-epicatechin). Figure S5: HPLC chromatogram at 520 nm of cornelian cherry juice (7—cyanidin-3-glucoside; 8—pelargonidine derivate). Figure S6: HPLC chromatogram at 280 nm of cornelian cherry juice (9—gallic acid). Figure S7: HPLC chromatogram at 250 nm of cornelian cherry juice (10—ellagic acid). Figure S8: HPLC chromatogram at 320 nm of cornelian cherry juice (5—chlorogenic acid). Figure S9: HPLC chromatogram at 245 nm of cornelian cherry juice (11—loganic acid; 12—cornuside). Figure S10: HPLC chromatogram at 520 nm of blackthorn juice (7—cyanidin-3-glucoside; 2—cyanidin-3-rutinoside). Figure S11: HPLC chromatogram at 360 nm of blackthorn juice (13—hyperoside). Figure S12: HPLC chromatogram at 320 nm of blackthorn juice (4—neochlorogenic acid).

**Author Contributions:** Conceptualization, D.R., M.A.L., J.Š., and M.K.; methodology, D.R. and M.K.; formal analysis, M.R., J.H., I.Ć., D.R., and M.K.; investigation, D.R., M.A.L., and J.Š.; data curation, M.R., J.H., I.Ć., D.R., and M.K.; writing—original draft preparation, D.R. and M.K.; writing—review and editing, M.A.L. and J.Š.; supervision, D.R. and M.K.; project administration, M.K.; funding acquisition, M.K. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study was funded and supported by Croatian Science Foundation under the grant number IP-2019-04-5749. Ina Ćorković acknowledges support from the Croatian Science Foundation program for Training New Doctoral Students (DOK-2020-01-4205).

**Data Availability Statement:** Data is contained within the article and Supplementary Materials.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## References

1. Louis, D.N.; Perry, A.; Wesseling, P.; Brat, D.J.; Cree, I.A.; Figarella-Branger, D.; Hawkins, C.; Ng, H.K.; Pfister, S.M.; Reifenberger, G.; et al. The 2021 WHO Classification of Tumors of the Central Nervous System: A Summary. *Neuro-Oncology* **2021**, *23*, 1231–1251. [CrossRef]
2. Muzyka, L.; Goff, N.K.; Choudhary, N.; Koltz, M.T. Systematic Review of Molecular Targeted Therapies for Adult-Type Diffuse Glioma: An Analysis of Clinical and Laboratory Studies. *Int. J. Mol. Sci.* **2023**, *24*, 10456. [CrossRef]
3. Thani, N.A.A.; Sallis, B.; Nuttall, R.; Schubert, F.R.; Ahsan, M.; Davies, D.; Purewal, S.; Cooper, A.; Rooprai, H.K. Induction of apoptosis and reduction of MMP gene expression in the U373 cell line by polyphenolics in *Aronia melanocarpa* and by curcumin. *Oncol. Rep.* **2012**, *28*, 1435–1442. [CrossRef]
4. Sánchez-Velázquez, O.A.; Cortés-Rodríguez, M.; Milán-Carrillo, J.; Montes-Ávila, J.; Robles-Bañuelos, B.; Santamaría del Ángel, A.; Cuevas-Rodríguez, E.O.; Rangel-López, E. Anti-oxidant and anti-proliferative effect of anthocyanin enriched fractions from two Mexican wild blackberries (*Rubus* spp.) on HepG2 and glioma cell lines. *J. Berry Res.* **2020**, *10*, 513–529. [CrossRef]
5. Thakkar, J.P.; Dolecek, T.A.; Horbinski, C.; Ostrom, Q.T.; Lightner, D.D.; Barnholtz-Sloan, J.S.; Villano, J.L. Epidemiologic and molecular prognostic review of glioblastoma. *Cancer Epidemiol. Biomarkers Prev.* **2014**, *23*, 1985–1996. [CrossRef]

6. Rooprai, H.K.; Christidou, M.; Murray, S.A.; Davies, D.; Selway, R.; Gullan, R.W.; Pilkington, G.J. Inhibition of Invasion by Polyphenols from Citrus Fruit and Berries in Human Malignant Glioma Cells In Vitro. *Anticancer Res.* **2021**, *41*, 619–633. [CrossRef]
7. Ramírez-Expósito, M.J.; Martínez-Martos, J.M. The Delicate Equilibrium between Oxidants and Antioxidants in Brain Glioma. *Curr. Neuropharmacol.* **2019**, *17*, 342–351. [CrossRef]
8. Stupp, R.; Mason, W.P.; van den Bent, M.J.; Weller, M.; Fisher, B.; Taphoorn, M.J.; Belanger, K.; Brandes, A.A.; Marosi, C.; Bogdahn, U.; et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N. Engl. J. Med.* **2005**, *352*, 987–996. [CrossRef]
9. Lamy, S.; Lafleur, S.; Bedard, V.; Moghrabi, A.; Barrete, S.; Gingras, D.; Béliveau, R. Anthocyanidins inhibit migration of glioblastoma cells: Structure-activity relationship and involvement of the plasmolytic system. *J. Cell Biochem.* **2007**, *100*, 100–111. [CrossRef]
10. Raucher, D. Tumor targeting peptides: Novel therapeutic strategies in glioblastoma. *Curr. Opin. Pharmcol.* **2019**, *47*, 14–19. [CrossRef]
11. Karachi, A.; Dastmalchi, F.; Mitchell, D.A.; Rahman, M. Temozolomide for immunomodulation in the treatment of glioblastoma. *Neuro Oncol.* **2018**, *20*, 1566–1572. [CrossRef] [PubMed]
12. Jiapaer, S.; Furuta, T.; Tanaka, S.; Kitabayashi, T.; Nakada, M. Potential Strategies Overcoming the Temozolomide Resistance for Glioblastoma. *Neurol. Med. Chir.* **2018**, *58*, 405–421. [CrossRef] [PubMed]
13. Tomar, M.S.; Kumar, A.; Srivastava, C.; Shrivastava, A. Elucidating the mechanisms of Temozolomide resistance in gliomas and the strategies to overcome the resistance. *Biochim. Biophys. Acta Rev. Cancer* **2021**, *1876*, 188616. [CrossRef] [PubMed]
14. Dhungel, L.; Rowsey, M.E.; Harris, C.; Raucher, D. Synergistic Effects of Temozolomide and Doxorubicin in the Treatment of Glioblastoma Multiforme: Enhancing Efficacy through Combination Therapy. *Molecules* **2024**, *29*, 840. [CrossRef] [PubMed]
15. Liu, X.; Hu, Y.; Xue, Z.; Zhang, X.; Liu, X.; Liu, G.; Wen, M.; Chen, A.; Huang, B.; Li, X.; et al. Valtrate, an iridoid compound in Valeriana, elicits anti-glioblastoma activity through inhibition of the PDGFRA/MEK/ERK signaling pathway. *J. Transl. Med.* **2023**, *21*, 147. [CrossRef] [PubMed]
16. Newman, D.J.; Cragg, G.M. Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. *J. Nat. Prod.* **2020**, *83*, 770–803. [CrossRef]
17. Vengoji, R.; Macha, M.A.; Batra, S.K.; Shonka, N.A. Natural products: A hope for glioblastoma patients. *Oncotarget* **2018**, *9*, 22194–22219. [CrossRef]
18. Reddy, L.A.; Odhav, B.; Bhoola, K.D. Natural products for cancer prevention: A global perspective. *Pharmacol. Ther.* **2003**, *99*, 1–3. [CrossRef] [PubMed]
19. Prietsch, R.F.; Monte, L.D.; Da Silva, F.A.; Beira, F.T.; Del Pino, F.A.B.; Campos, V.F.; Collares, T.; Pinto, L.S.; Spanevello, R.M.; Gamaro, G.D.; et al. Genistein induces apoptosis and autophagy in human breast MCF-7 cells by modulating the expression of proapoptotic factors and oxidative stress enzymes. *Mol. Cell Biochem.* **2014**, *390*, 235–242. [CrossRef]
20. Liu, Y.; Jiang, Y.G. Podocalyxin promotes glioblastoma multiforme cell invasion and proliferation via β-catenin signaling. *PLoS ONE* **2014**, *28*, e111343. [CrossRef]
21. Ouyang, Z.; Xu, G. Antitumor Effects of Sweroside in Human Glioblastoma: Its Effects on Mitochondrial Mediated Apoptosis, Activation of Different Caspases, G0/G1 Cell Cycle Arrest and Targeting JNK/P38 MAPK Signal Pathways. *J. BUON* **2019**, *24*, 2141–2146. [PubMed]
22. Leung, H.W.; Lin, C.J.; Hour, M.J.; Yang, W.H.; Wang, M.Y.; Lee, H.Z. Kaempferol induces apoptosis in human lung non-small carcinoma cells accompanied by an induction of antioxidant enzymes. *Food Chem. Toxicol.* **2007**, *45*, 2005–2013. [CrossRef] [PubMed]
23. Kamei, H.; Kojima, T.; Hasegawa, M.; Koide, T.; Umeda, T.; Yukawa, T.; Terabe, K. Suppression of tumor cell growth by anthocyanins in vitro. *Cancer Investig.* **1995**, *13*, 590–594. [CrossRef] [PubMed]
24. Reddy, M.K.; Alexander-Lindo, R.L.; Nair, M.G. Relative inhibition of lipid peroxidation, cyclooxygenase enzymes, and human tumor cell proliferation by natural food colors. *J. Agric. Food Chem.* **2005**, *53*, 9268–9273. [CrossRef] [PubMed]
25. Michaud-Levesque, J.; Bousquet-Gagnon, N.; Beliveau, R. Quercetin abrogates IL-6/STAT3 signaling and inhibits glioblastoma cell line growth and migration. *Exp. Cell Res.* **2012**, *318*, 925–935. [CrossRef] [PubMed]
26. Ndongwe, T.; Witika, B.A.; Mncwangi, N.P.; Poka, M.S.; Skosana, P.P.; Demana, P.H.; Summers, B.; Siwe-Noundou, X. Iridoid Derivatives as Anticancer Agents: An Updated Review from 1970–2022. *Cancers* **2023**, *15*, 770. [CrossRef]
27. Allen, M.; Bjerke, M.; Edlund, H.; Nelander, S.; Westermark, B. Origin of the U87MG glioma cell line: Good news and bad news. *Sci. Transl. Med.* **2016**, *8*, 354re3. [CrossRef]
28. Pevna, V.; Wagnières, G.; Huntosova, V. Autophagy and Apoptosis Induced in U87 MG Glioblastoma Cells by Hypericin-Mediated Photodynamic Therapy Can be Photobiomodulated with 808 nm Light. *Biomedicines* **2021**, *9*, 1703. [CrossRef]
29. Mousavi, M.; Koosha, F.; Neshastehriz, A. Chemo-radiation therapy of U87-MG glioblastoma cells using SPIO@AuNP-Cisplatin-Alginate nanocomplex. *Heliyon* **2023**, *9*, e13847. [CrossRef]
30. Di Cintio, F.; Dal Bo, M.; Baboci, L.; De Mattia, E.; Polano, M.; Toffoli, G. The Molecular and Microenvironmental Landscape of Glioblastomas: Implications for the Novel Treatment Choices. *Front. Neurosci.* **2020**, *14*, 603647. [CrossRef]
31. Nguyen, H.-M.; Guz-Montgomery, K.; Lowe, D.B.; Saha, D. Pathogenetic Features and Current Management of Glioblastoma. *Cancers* **2021**, *13*, 856. [CrossRef] [PubMed]

32. Tsai, C.-Y.; Ko, H.-J.; Huang, C.-Y.F.; Lin, C.-Y.; Chiou, S.-J.; Su, Y.-F.; Lieu, A.-S.; Loh, J.-K.; Kwan, A.-L.; Chuang, T.-H.; et al. Ionizing Radiation Induces Resistant Glioblastoma Stem-Like Cells by Promoting Autophagy via the Wnt/β-Catenin Pathway. *Life* **2021**, *11*, 451. [CrossRef] [PubMed]
33. Singleton, V.L.; Rossi, J.A. Colorimetry of total phenolics with phosphomolybdic-phosphotonutric acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158. [CrossRef]
34. Prior, R.L.; Fan, E.; Ji, H.; Howell, A.; Nio, C.; Payne, M.J.; Reed, J. Multi-laboratory validation of a standard method for quantifying proanthocyanidins in cranberry powders. *J. Sci. Food Agric.* **2010**, *90*, 1473–1478. [CrossRef]
35. Giusti, M.M.; Wrolstad, R.E. Characterization and Measurement of Anthocyanins by UV-Visible Spectroscopy. In *Current Protocols in Food Analytical Chemistry Current Protocols*; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2001.
36. Arnao, M.B.; Cano, A.; Acosta, M. The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chem.* **2001**, *73*, 239–244. [CrossRef]
37. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT* **1995**, *28*, 25–30. [CrossRef]
38. Benzie, I.F.F.; Strain, J.J. The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant Power": The FRAP assay. *Anal. Biochem.* **1996**, *239*, 70–76. [CrossRef] [PubMed]
39. Apak, R.; Güçlü, K.; Ozyürek, M.; Karademir, S.E. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *J. Sci. Food Agric.* **2004**, *52*, 7970–7981. [CrossRef]
40. Pambianchi, E.; Hagenberg, Z.; Pecorelli, A.; Grace, M.; Therrien, J.-P.; Lila, M.A.; Valacchi, G. Alaskan bog blueberry (Vaccinium uliginosum) extract as an innovative topical approach to prevent UV-induced skin damage. *Cosmetics* **2021**, *8*, 112. [CrossRef]
41. Može, Š.; Polak, T.; Gašperlin, L.; Koron, D.; Vanzo, A.; Poklar Ulrih, N.; Abram, V. Phenolics in Slovenian Bilberries (*Vaccinium myrtillus* L.) and Blueberries (*Vaccinium corymbosum* L.). *J. Agric. Food Chem.* **2011**, *59*, 6998–7004. [CrossRef]
42. Buljeta, I.; Pichler, A.; Šimunović, J.; Kopjar, M. Polyphenols and antioxidant activity of citrus fiber/blackberry juice complexes. *Molecules* **2021**, *26*, 4400. [CrossRef] [PubMed]
43. Mosmann, T. Rapid colorimetric assay for cellular proliferation and viability: Application to the proliferation and viability of murine macrophages. *J. Immunol. Methods* **1983**, *65*, 55–63. [CrossRef] [PubMed]
44. Kopjar, M.; Raucher, D.; Lila, M.A.; Šimunović, J. Anti-Glioblastoma Potential and Phenolic Profile of Berry Juices. *Processes* **2024**, *12*, 242. [CrossRef]
45. Gálvez, M.; Martín-Cordero, C.; Ayuso, M.J. Iridoids as DNA Topoisomers I Poisons. *J. Enzym. Inhib. Med. Chem.* **2005**, *20*, 389–392. [CrossRef] [PubMed]
46. Dinda, B.; Roy Chowdhury, D.; Mohanta, B.C. Naturally Occurring Iridoids, Secoiridoids and Their Bioactivity. An Updated Review, Part 3. *Chem. Pharm. Bull.* **2009**, *57*, 765–796. [CrossRef] [PubMed]
47. Lou, C.; Zhu, Z.; Xu, X.; Zhu, R.; Sheng, Y.; Zhao, H. Picroside II, an Iridoid Glycoside from Picrorhiza Kurroa, Suppresses Tumor Migration, Invasion, and Angiogenesis in Vitro and in Vivo. *Biomed. Pharmacother.* **2019**, *120*, 109494. [CrossRef] [PubMed]
48. Wang, C.; Xin, P.; Wang, Y.; Zhou, X.; Wei, D.; Deng, C.; Sun, S. Iridoids and Sfingolipids from Hedyotis Diffusa. *Fitoterapia* **2018**, *124*, 152–159. [CrossRef] [PubMed]
49. Villasenor, I. Bioactivities of Iridoids. *Antiinflamm Antiallergy Agents Med. Chem.* **2008**, *6*, 307–314. [CrossRef]
50. Viljoen, A.; Mncwangi, N.; Vermaak, I. Anti-Inflammatory Iridoids of Botanical Origin. *Curr. Med. Chem.* **2012**, *19*, 2104–2127. [CrossRef]
51. Sozański, T.; Kucharska, A.Z.; Szumny, A.; Magdalan, J.; Bielska, K.; Merwid-Ląd, A.; Woźniak, A.; Dzimira, S.; Piórecki, N.; Trocha, M. The protective effect of the *Cornus mas* fruits (cornelian cherry) on hypertriglyceridemia and atherosclerosis through PPARα activation in hypercholesterolemic rabbits. *Phytomed* **2014**, *21*, 1774–1784. [CrossRef]
52. Kim, N.Y.; Ha, I.J.; Um, J.-Y.; Kumar, A.P.; Sethi, G.; Ahn, K.S. Loganic acid regulates the transition between epithelial and mesenchymal-like phenotypes by alleviating MnSOD expression in hepatocellular carcinoma cells. *Life Sci.* **2023**, *317*, 121458. [CrossRef] [PubMed]
53. Radbeha, Z.; Asefi, N.; Hamishehkar, H.; Roufegarinejad, L.; Pezeshki, A. Novel carriers ensuring enhanced anti-cancer activity of *Cornus mas* (cornelian cherry) bioactive compounds. *Biomed. Pharmacother.* **2020**, *125*, 109906.
54. Tang, J.; Oroudjev, E.; Wilson, L.; Ayoub, G. Delphinidin and cyanidin exhibit antiproliferative and apoptotic effects in MCF7 human breast cancer cells. *Integr. Cancer Sci. Therap.* **2015**, *2*, 82–86.
55. Jing, N.; Song, J.; Liu, Z.; Wang, L.; Jiang, G. Glycosylation of anthocyanins enhances the apoptosis of colon cancer cells by handicapping energy metabolism. *BMC Complement. Med. Ther.* **2020**, *20*, 312. [CrossRef] [PubMed]
56. Chen, P.-N.; Chu, S.-C.; Chiou, H.-L.; Kuo, W.-H.; Chiang, C.-L.; Hsieh, Y.-S. Mulberry anthocyanins, cyanidin 3-rutinoside and cyanidin 3-glucoside, exhibited an inhibitory effect on the migration and invasion of a human lung cancer cell line. *Cancer Lett.* **2006**, *235*, 248–259. [CrossRef] [PubMed]
57. Kang, S.-Y.; Seeram, N.P.; Nair, M.G.; Bourquin, L.D. Tart cherry anthocyanins inhibit tumor development in $Apc^{Min}$ mice and reduce proliferation of human colon cancer cells. *Cancer Lett.* **2003**, *194*, 13–19. [CrossRef] [PubMed]
58. Zhang, F.-J.; Yang, J.-Y.; Mou, Y.-H.; Sun, B.-S.; Ping, Y.-F.; Wang, J.-M.; Bian, X.-W.; Wu, C.-F. Inhibition of U-87 human glioblastoma cell proliferation and formyl peptide receptor function by oligomer procyanidins (F2) isolated from grape seeds. *Chem. Biol. Interact.* **2009**, *179*, 419–429. [CrossRef] [PubMed]

59. Ferguson, P.J.; Kurowska, E.M.; Freeman, D.J.; Chambers, A.F.; Koropatnick, J. In vivo inhibition of growth of human tumor lines by flavonoid fractions from cranberry extract. *Nutr. Cancer* **2006**, *56*, 86–94. [CrossRef] [PubMed]
60. Karakas, N.; Okur, M.E.; Ozturk, I.; Ayla, S.; Karadag, A.E.; Çiçek Polat, D. Antioxidant Activity of Blackthorn (*Prunus spinosa* L.) Fruit Extract and Cytotoxic Effects on Various Cancer Cell Lines. *Medeni. Med. J.* **2019**, *34*, 297–304.
61. Roginsky, V.; Lissi, E.A. Review of methods to determine chain-breaking antioxidant activity in food. *Food Chem.* **2005**, *92*, 235–254. [CrossRef]
62. Abirami, A.; Sinsinwar, S.; Rajalakshmi, P.; Brindha, P.; Rajesh, Y.B.R.D.; Vadivel, V. Antioxidant and cytoprotective properties of loganic acid isolated from seeds of Strychnos potatorum L. against heavy metal induced toxicity in PBMC model. *Drug Chem. Toxicol.* **2022**, *45*, 239–249. [CrossRef] [PubMed]
63. Shahidi, F.; Zhong, Y. Measurement of antioxidant activity. *J. Funct. Foods* **2015**, *18*, 757–781. [CrossRef]
64. Prior, R.L.; Wu, X.; Schaich, K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.* **2005**, *53*, 4290–4302. [CrossRef] [PubMed]
65. Pinelo, M.; Manzocco, L.; Nunez, M.J.; Nicoli, M.C. Interaction among phenols in food fortification: Negative synergism on antioxidant capacity. *J. Agric. Food Chem.* **2004**, *52*, 1177–1180. [CrossRef] [PubMed]
66. Hang, D.T.N.; Hoa, N.T.; Bich, H.N.; Mechler, A.; Vo, Q.V. The hydroperoxyl radical scavenging activity of natural hydroxybenzoic acids in oil and aqueous environments: Insights into the mechanism and kinetics. *Phytochemistry* **2022**, *201*, 113281. [CrossRef]
67. Biela, M.; Kleinová, A.; Klein, E. Phenolic acids and their carboxylate anions: Thermodynamics of primary antioxidant action. *Phytochemistry* **2022**, *200*, 113254. [CrossRef] [PubMed]
68. Haizhou, W.; Oliveira, G.; Lila, M.A. Protein-binding approaches for improving bioaccessibility and bioavailability of anthocyanins. *Compr. Rev. Food Sci. Food Saf.* **2023**, *22*, 333–354.
69. Hribar, U.; Poklar Ulrih, N. The Metabolism of Anthocyanins. *Curr. Drug Metab.* **2014**, *15*, 3–13. [CrossRef] [PubMed]
70. Janle, E.M.; Lila, M.A.; Grannan, M.; Wood, L.; Higgins, A.; Yousef, G.G.; Rogers, R.B.; Kim, H.; Jackson, G.S.; Weaver, C. Method for evaluating the potential of 14C labeled plant polyphenols to cross the blood-brain barrier using accelerator mass spectrometry. *Nucl. Instrum. Methods Phys. Res.* **2010**, *268*, 1313–1316. [CrossRef]
71. Olędzka, A.; Cichocka, K.; Wolieński, K.; Melzig, M.F.; Czerwińska, M.E. Potentially Bio-Accessible Metabolites from an Extract of *Cornus mas* Fruit after Gastrointestinal Digestion In Vitro and Gut Microbiota Ex Vivo Treatment. *Nutrients* **2022**, *14*, 2287. [CrossRef]
72. Jugran, A.K.; Rawat, S.; Bhatt, I.D.; Rawal, R.S. Valeriana jatamansi: An herbaceous plant with multiple medicinal uses. *Phytother. Res.* **2019**, *33*, 482–503. [CrossRef] [PubMed]
73. Qu, K.; Zhao, L.; Luo, X.; Zhang, C.; Hou, P.; Bi, K.; Chen, X. An LC-MS method for simultaneous determination of five iridoids from Zhi-zi-chi Decoction in rat brain microdialysates and tissue homogenates: Towards an in depth study for its antidepressive activity. *J. Chromatogr. B* **2014**, *965*, 206–215. [CrossRef] [PubMed]
74. Ramakrishna, R.; Bhateria, M.; Singh, R.; Puttrevu, S.K.; Bhatta, R.S. Plasma pharmacokinetics, bioavailability and tissue distribution of agnuside following peroral and intravenous administration in mice using liquid chromatography tandem mass spectrometry. *J. Pharm. Biomed. Anal.* **2016**, *125*, 154–164. [CrossRef]
75. Wang, C.; Gong, X.; Bo, A.; Zhang, L.; Zhang, M.; Zang, E.; Zhang, C.; Li, M. Iridoids: Research Advances in Their Phytochemistry, Biological Activities, and Pharmacokinetics. *Molecules* **2020**, *25*, 287. [CrossRef] [PubMed]